REMARKS

Pending claims 45 – 48 and 66 – 114.

Cancelled claims 1 – 44 and 49 – 65.

In Response to the Office Action of November 30, 2004, Applicants amended independent claims 45 and 91 and introduced a new independent claim 111, all of which are fully supported by the original specification and drawings. For example, the parent US application 09/005,985 discloses:

> FIG. 29 is a partial sectional view of a cartridge adapted for low volume hybridization of high density oligonucleotide arrays. (page 7, lines 16 – 18; emphasis added)
>
> FIG. 41 illustrates a low volume hybridization system having a movable pneumatically-controlled wall.
>
> FIG. 42 illustrates a low volume hybridization system having a movable pneumatically-controlled pivoting wall. (page 8, lines 7 – 10)

On page 93, line 11 through page 95, line 33, the specification discloses the following:

> As is seen in FIG. 29 a hybridization system 3100 includes a base 3102 that defines a hybridization chamber 3122 with a pneumatic port 3110 and a fluidic port 3111. The probe array 3112 is mounted to base 3102 and a thermal control block 3124 for controlling the temperature of probe array 3112 during hybridization. According to the present invention, a composite porous membrane 3120 is positioned a relatively small distance (e.g., 10 to 100 um) from probe array 3112 to create a smaller chamber 3122 therebetween. The porous membrane 3120 preferably comprises a sandwich of hydrophobic material, such as Versapore 200 from Gelman associates, and a thin membrane with neutral wetting properties, such as particle-track etched polycarbonate from Poretics.
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> After the target solution is introduced into hybridization chamber 3122, complete filling is effectively ensured by pulling a vacuum on pneumatic port 3110. The pneumatic port 3110 is then pressurized to inject a high density of bubbles substantially uniformly into hybridization chamber 3122. The bubbles provide mixing by expanding, coalescing, and impacting the oligonucleotide array 3112. Further mixing may be induced by pulling a vacuum on port 3110 and withdrawing the bubbles from the chamber. Alternatively, injecting and withdrawing gas from the hybridization chamber results in aggressive uniform convection to the entire oligonucleotide array surface.
>
> Current hybridization chambers typically have a volume of 250 µl. However, lower volume hybridization chambers would provide greater sensitivity and shorter assay time. Unfortunately, when attempting to design hybridization

chambers having very small height dimensions, surface tension and wetting effects become problematic, thereby making the control of fluids and bubbles within the chamber difficult, especially when the chamber height is reduced below 0.5 mm. Specifically, capillary pressures increase inversely with the chamber height, so that a 0.1 mm high chamber with non-wetting walls corresponds to 0.2 psi for water. Pressures in this range are typically sufficient to frustrate fluid control.

The low volume hybridization systems of the present invention, as set forth herein, are adapted to operate at volumes in the range of 0.1 to 100 µl, and more preferably in the range of 1 to 20 µl, and most preferably, in the range of 5 to 10 µl.

FIG. 41 illustrates an embodiment of a low volume hybridization system 4800 which avoids the above limitations. Specifically, hybridization system 4800 includes a hybridization chamber 4802 and pneumatic ports 4804 and 4806. A probe array 4812 is mounted to base 4803. A flexible diaphragm 4820 is included and is addressed by pneumatic ports 4804 and 4806 such that movement of flexible diaphragm 4820 operates to decrease the height of hybridization chamber 4802 such that the chamber volume can be expanded for draining and filling operations and contracted for hybridization. Draining and filling of chamber 4802 is accomplished by simultaneously applying a pressure or a vacuum to pneumatic ports 4804 and 4806. Mixing in chamber 4802 during the hybridization stage can be accomplished by alternatively applying pressures or vacuums to pneumatic ports 4804 and 4806, thus causing separate portions 4820A and 4820B of diaphragm 4820 [proximal pneumatic ports 4804 and 4806, respectively] to flex in a manner such that fluid is squeezed back and forth within the hybridization chamber as the chamber height above diaphragm portions 4820A and 4820B is varied.

FIG. 42 illustrates an alternative embodiment of a very low volume hybridization system 4900 which includes a hybridization chamber 4902 and a pneumatic port 4904. Probe array 4912 is mounted to base 4903. A flexible diaphragm 4920 mounted to a rigid plate 4922 is also included. Flexible diaphragm 4920 extends fully across the top and thereby seals pressure chamber 4905. Rigid plate 4922 has a hinged end 4923 and a free end 4925. Accordingly, rigid plate 4922 pivots about hinged end 4923 as a pressure differential is applied to pneumatic port 4904. Specifically, as the pressure in pressure chamber 4905 is decreased, rigid plate 4922 pivots downwardly at its free end 4925. Correspondingly, as the pressure in pressure chamber 4905 is increased, rigid plate 4922 pivots upwardly at its free end 4925. As such, the dimension of hybridization chamber 4902 can easily be varied by tilting rigid plate 4922 by applying a pressure differential at pneumatic port 4904. Due to the effects of surface tension, hybridization fluid 4930 will tend to collect at the narrow end of hybridization chamber 4902, as shown. Therefore, decreasing the volume of hybridization chamber 4902 by tilting rigid plate 4922 upwardly will cause the fluid to spread across the surface of the flexible diaphragm. As a consequence, repetitive application of a pressure differential in chamber 4902 will cause the rigid plate 4922 to tilt upwardly and downwardly will cause mixing in the fluid as it repetitively spreads out and then retracts across the diaphragm surface. In addition, upward tilting of rigid plate 4922 also reduces the volume of the hybridization chamber 4902. Draining and filling can be accomplished by applying a vacuum to pneumatic port 4904.

> In another embodiment, motion of the membrane is provided using forces other than pneumatic (e.g., electrostatic, magnetic, or piezoelectric). For example, plate 4925 is metallic and a moving magnetic field moves the plate.

Therefore, based on the above-cited description, independent claims 45, 91 and 111 are fully supported by the original specification. The amended dependent claims 74 and 99 are also supported by the above-cited description.

In the Office Action of November 30, 2004, the Examiner rejected claims 66 -110 under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. Applicants respectfully disagree with this rejection and submit that they clearly complied with the written description requirement, with respect to claims 66 – 110, as viewed by a person of ordinary skill in this art.

When rejecting claims 66 -110 under 35 U.S.C. §112, first paragraph, the Examiner assumed a very low level of skill for a person of ordinary skill in the art since the Examiner assumed that such person would not be able to read the specification (i.e., a single document) and combine various disclosed embodiments (and if needed make them smaller). On the other hand, when rejecting claims 45 – 48 and 66 -110 under 35 U.S.C. §103, the Examiner assumed a very high level of skill for the person of ordinary skill in the art since the Examiner assumed that such person would be able to combine several separate documents to arrive at the claimed invention. Notably, with respect to claims 72, 73, 75-77, 79, 97, 98, 100, 101 and 103, the Examiner assumed that such person would be able to combine *three* separate documents (i.e., Andrevski, Southgate and Wainwright) to arrive at the claimed invention. Regarding claims 78, 80-86, 102 and 104-110, the Examiner again assumed that such person would be able to combine *three* separate documents (i.e., Andrevski, Southgate and Schnipelsky) to arrive at the claimed invention. This is inconsistent since there is only one level of ordinary skill in the art to be applied under 35 U.S.C. §112, first paragraph, and to be applied under 35 U.S.C. §103.

As explained below, when filing the priority application, Applicants were fully in possession of the invention claimed in claims 66 –114 and also provided enabling

disclosure for these claims. The Examiner seemed to acknowledge that the subject matter of claims 45 – 48 is fully enabled. However, when rejected, independent claim 91 includes basically all limitations of claim 45 in combination with a processing chamber connectable to the reaction chamber (recited in claims 45) and constructed for exchanging fluids with the reaction chamber. The present application discloses numerous embodiments with different chambers, valves and vents. It is truly inconceivable that, *after reading the present specification*, a person of ordinary skill in the art would not be able to couple the hybridization chamber recited in claim 45 with a processing chamber.

Applicants respectfully submit that all elements claimed in claims 66 – 90 were originally disclosed in the priority document that teaches the various claimed combination together with the low volume hybridization device of claim 45. Three embodiments of the low volume hybridization device are described on page 93, line 11 through page 95, line 33 and in Figs. 29, 41 and 42, as filed in the US Application 09/005,985.

The entire US Application 09/005,985 is directed to various aspects of a *miniaturized device*. The specification introduces on page 3 line 33 though page 4, line 13, this subject matter for a person of ordinary skill in the art as follows: "Interest has been growing in the fabrication of microfluidic devices. Typically, advances in the semiconductor manufacturing arts have been translated to the fabrication of micromechanical structures, e.g., micropumps, microvalves, and the like, and microfluidic devices including miniature chambers and flow passages. A number of researchers have attempted to employ these microfabrication techniques in the miniaturization of some of the processes involved in genetic analysis in particular. For example, published PCT Application No. WO 94/05414, to Northrup and White, incorporated herein by reference in its entirety for all purposes, reports an integrated micro-PCR apparatus for collection and amplification of nucleic acids from a specimen. However, there remains a need for an apparatus which combines the various

processing and analytical operations involved in nucleic acid analysis. The present invention meets these and other needs."

The present specification made it clear that although described in terms of individual devices, the present specification is directed to the combination of the described devices. On page 9 line 26, the present specification states:

> It is a general object of the present invention to provide a miniaturized integrated nucleic acid diagnostic devices and systems incorporating these devices. The devices of the invention are generally capable of performing one or more sample acquisition and preparation operations, as may be integrated with one or more sample analysis operations. For example, the devices can integrate several or all of the operations involved in sample acquisition and storage, sample preparation and sample analysis, within a single, miniaturized, integrated unit. The devices are useful in a variety of applications and most notably, nucleic acid based diagnostic applications and de novo sequencing applications.
>
> The devices of the invention will typically be one component of a larger diagnostic system, which further includes reader device for scanning and obtaining the data from the device, and a computer based interface for controlling the device and/or interpretation of the data derived from the device.
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> To carry out their primary functions, one embodiment of the devices of the invention will typically incorporate a plurality of distinct reaction chambers for carrying out the sample acquisition, preparation and analysis operations. In particular, a sample to be analyzed is introduced into the device whereupon it will be delivered to one of these distinct reaction chambers which are designed for carrying out a variety of reactions as a prelude to analysis of the sample. These preparative reactions generally include, e.g., sample extraction, PCR amplification, nucleic acid fragmentation and labeling, extension reactions, transcription reactions and the like.

Furthermore, on page 117, line 16, the present specification states:

> Although generally described in terms of individual devices, it will be appreciated that multiple devices may be provided in parallel to perform analyses on a large number of individual samples. Because the devices are miniaturized, reagent and/or space requirements are substantially reduced. Similarly, the small size allows automation of sample introduction process using, e.g., robot samplers and the like.

On page 88 lines 16 - 25, in connection with microreactive chambers, the specification discloses the following: "Miniaturization provides opportunities for enhanced uniformity and rapid cycling. Smaller reaction chambers will tend to be more isothermal and cool faster than their larger-volume counterparts. A thermal cycling device generally should meet two competing criteria: (1) maintain wall temperatures

without excessive heat dissipation; and (2) have the ability to change temperature rapidly. According to the present invention, this is accomplished by providing arrays of separately addressable heaters over an insulating layer that is in contact with a cooler (e.g. thermoelectric cooler)."

On page 91 line 27, the specification discloses: "According to the present invention, a miniaturized sample-preparation system comprises chambers for reagent storage, reactions, and/or hybridization. The chambers are preferably defined in an injection-molded package that forms a cartridge (as discussed above in previous embodiments). Similar to above, movement of liquid between the chambers is carried out by pneumatic signals provided to the cartridge by a base instrument..."

A person of ordinary skill in the art would not be able to combine the hybridization chamber recited in claim 45 with various structural elements needed for storage, reactions, and/or hybridization. The specification clearly teaches on page 92, line 30 through page 92, line 4 the following:

> A nucleic acid sample, (target) requirements for hybridization can typically be reduced by decreasing the hybridization chamber volume. Hybridization is currently carried out in a cartridge with an internal volume of about 250 µl and a 10 nM target, requiring about 2.5 pmoles. By decreasing the chamber volume to about 10 µl, only about 100 fmoles of target is required to maintain a 10 nM concentration. Typically, aggressive mixing is necessary to achieve rapid and reproducible hybridization with sufficient signal and discrimination. One method of reducing the chamber volume is to decrease the distance between the oligonucleotide probe array and the opposite surface of the cartridge.

All claim elements are disclosed in the pending application. For example, the subject matter of claim 66, 68, 68 and 70 are clearly disclosed in connection with Fig. 23. The subject matter of claim 71 - 75 is disclosed on pages 85 and 86, and the subject matter of claim 76, 80, 82 and 83 is disclosed on page 87. The subject matter of claim 75 – 77 and 79 is disclosed on page 72, claim 86 on page 91, claim s 87, 88 and 92 in connection with Figs. 6 – 6C, The subject matter of claim 93 and 94 is disclosed on pages 120, claims 84, 89 and 108 on pages 34 and 35, and claim 90 on page 58.

In summary, all claim elements claimed in claims 66 –114 are disclosed in the present specification. Claims 112, 113 and 114 correspond to original claims 46, 47 and 48.

The Examiner also cited *Ex parte Ohshiro* (14 USPQ2d 1750) when making the rejections under 35 U.S.C. §112, first paragraph. However, *Ex parte Ohshiro* is not applicable to the present case. In *Ex parte Ohshiro* the claimed "piston" species were not disclosed at all in the specification. However, in the present case, the claimed elements are disclosed in the present specification.

The Examiner also rejected claims 45-48, 71, 78-96 and 102-110 under 35 U.S.C. §103(a) as being unpatentable over US Pat. 5,882,903 to Andrevski et al. in view of US Pat. 5,863,502 to Southgate et al. The Examiner also rejected claims 72, 73, 75-77, 79, 97, 98, 100, 101 and 103 under 35 U.S.C. §103(a) as being unpatentable over US Pat. 5,882,903 to Andrevski et al. in view of US Pat. 5,863,502 to Southgate et al. taken further in view of US Pat. 5,876,918 to Wainwright et al. The Examiner also rejected claims 78, 80-86, 102 and 104-110 are rejected under 35 U.S.C. §103(a) as being unpatentable over US Pat. 5,882,903 to Andrevski et al. in view of US Pat. 5,863,502 to Southgate et al. taken further in view of US Pat. 5,229,297 to Schnipelsky et al. Applicants respectfully disagree with the above rejections if again applied to the amended claims.

The invention claimed in the independent claims is directed to a low-volume hybridization device including a base, a pressure chamber, and a reaction chamber. The reaction chamber is bound by a flexible diaphragm separating the pressure chamber from the reaction chamber. The hybridization device is constructed for mounting a probe array to the base thereby disposing the probe surface inside the reaction chamber. The flexible diaphragm forms an opposing surface to the probe surface. The pressure chamber is constructed for application of pressure or vacuum to the flexible diaphragm thereby changing the distance between the array surface and the diaphragm surface to enable fluid displacement in the reaction chamber for achieving hybridization.

US Pat. 5,882,903 to Andrevski et al. alone, or in combination with US Pat. 5,863,502 to Southgate et al. does not disclose the low volume hybridization device claimed in independent claims 45, 91 and 111. In US Pat. 5,882,903, Andrevski discloses various reaction chambers, but does not disclose any probe array, as admitted by the Examiner. In column 24, line 66 to column 25, line 13, Southgate discloses a hybridization probe (or two or more hybridization probes) located on membranes inside detection channels 295 shown in Figs. 15A and 15B. Importantly, Andrevski in combination with Southgate provide no teaching regarding displacing fluid in a hybridization chamber by moving a diaphragm and thus changing the volume for the purpose of hybridization of the probe array.

US Pat. 5,882,903 to Andrevski et al. discloses a small volume reaction chamber 250 (or chambers 250A – 250C) designed for forcing fluids in and out. These chambers are shown in Figs. 1A – 5. Specifically, in connection with Figs. 4A, 4B and 4C, Andrevski discloses:

> FIGS. 4A, 4B and 4C illustrate a mechanism having upper auxiliary block 300A and lower auxiliary block 300B for forcing fluids into the chamber 250, wherein the assay system has any suitable geometry and structure 300 is an embodiment of a fluid impeller. Upper block 300A is honeycombed with passageways including upper gas inlet/outlet 310A, upper manifold 320A and a plurality of upper pressurized channels 321A. Upper channels 321A exit adjacent to the surface of upper membrane 241. The corresponding structures of the symmetrical lower block 300B are correspondingly numbered using the suffix "B" instead of "A". In FIG. 4B, gas pressure has been applied through upper gas inlet/outlet 310A and lower gas inlet/outlet 310B, so that gas exiting upper and lower pressurized channels 321A and 321B forces upper and lower membranes 241 and 242 together, thereby forcing fluid from chamber 250, thus providing explanation of the aforementioned fluid impeller embodiment. In FIG. 4C, a vacuum applied to gas inlet/outlets 310A and 310B creates suction at upper and lower channels 321A and 321B, causing the upper and lower membranes 241 and 242 to adhere to blocks 300A and 300B, respectively, thereby pulling upper and lower membranes 241 and 242 apart and partially evacuating the invention. The partial vacuum in chamber 250 helps draw fluid into the invention through one of first or second fluid exchange channels 231 or 232. (col. 5 lines 31 – 56)

Andrevski discloses changing volume of a reaction chamber. However, there is no teaching in Andrevski regarding mounting a probe array to the base of a hybridization device so that there is a probe array surface disposed inside a reaction chamber and in

combination with a flexible diaphragm being disposed over the array surface for the purpose of changing the volume of the reaction chamber by changing the distance between the array surface and the diaphragm surface. **NB:** Also, any probe of Southgate (or a probe array) would have to be located between membranes 241 and 242 (shown in Figs. 4A – 4C of Andrevski) or would need to be mounted onto the membranes, but such probe would not be mounted on the base (as claimed).

In US Pat. 5,863,502, Southgate discloses, in col. 24, line 25 through col. 25 line 4, the following:

> In a preferred embodiment, the cassette has one or more detection channels 295. One such detection channel 295 is illustrated in FIGS. 15A and 15B. It is made up of a number of fibers 297, which together preferably transmit at least about 50% of light of a wavelength useful in the detection procedure, confined to the detection channel 295. The fibers 297 can be bound in place for instance by cementing or crimping. The fibers 297 can be fabricated of glass or suitably transparent plastics. The fibers 297 are preferably between about 5 μm and about 50 μm in diameter, more preferably about 20 μm. The detection channel typically has a width and depth of no more than about 3,000 μm, preferably between about 200 μm and about 1,000 μm. Microchannels between the fibers 297 allow liquid to flow through the detection channel 295. A detection-mediating molecule is bound to the fibers 297. Preferably the detection-mediating molecule is an oligonucleotide that hybridizes with the nucleic acid to be amplified in a nucleic acid amplification reaction and the nucleic acid amplification reaction utilizes primers having a detectable moiety. The detection-mediating molecules are bound to the fibers 297 by known methods. Preferably, discrete bands on the fibers such as first band 296A, second band 296B and third band 296C have separate detection-mediating molecules, which could be, for instance, designed to detect two separate species to be amplified in a nucleic acid amplification reaction and to provide a control for non-specific hybridizations. To manufacture the banding pattern of bound molecules, oligonucleotide synthesis procedures that utilize photo-cleavable protecting groups and masks to protect certain bands 296 from photocleavage can be used. Such synthesis procedures are described in U.S. Pat. No. 5,424,186 (Fodor et al.). The instrument 900 is preferably designed to provide heat control at the detection channels 295 for conducting hybridization reactions. In a preferred embodiment, the sides 298 of the detection channel 295 are coated with a reflective coating so that light incident from above will reflect and twice pass through the detection channel 295. Such a reflective coating is provided by metalizing, for instance using a sputtering or evaporation process.
>
> Alternatively, the detection channels 295 contain membranes 299 (not shown), such as a nylon membrane, to which a hybridization probe has been bound. If two or more hybridization probes are used, they are each bound to a specific region of the membranes 299 using "dot blot" procedures such as are described in Bugawan et al...

Therefore, the combination of Andrevski and Southgate does not disclose a hybridization device where a probe array is mounted to the base in a manner that a probe array surface is disposed inside a reaction chamber. Andrevski and Southgate do not disclose a flexible diaphragm separating the pressure chamber from the reaction chamber and constructed to change the volume of the reaction chamber by changing the distance between the array surface and the diaphragm surface. Claim 111 also recites the hybridization device being constructed to introduce bubbles into the reaction chamber to provide for mixing at the array surface to enable fluid displacement in the reaction chamber for achieving hybridization of the probe array.

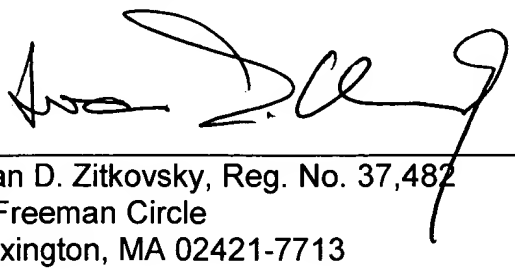
Therefore, independent claims 45, 91 and 111 are clearly patentable over the combination of Andrevski and Southgate. Dependent claims 46-48, 66-90, 92 – 110 and 112 – 114 include additional combinations of novel features.

The Examiner also rejected claims 74 and 99 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1 and 2 of U.S. Patent No. 6,168,948 in view of Andrevski et al. and Southgate et al. Applicants amended claims 74 and 99 to overcome this rejection.

Therefore, all pending claims are now in condition for allowance and such action is respectfully solicited. Should there be any outstanding issue left, the Examiner is respectfully invited to call the undersigned at the telephone number below.

Please charge all PTO fees to the Deposit Account No. 01-0431.

Respectfully submitted,



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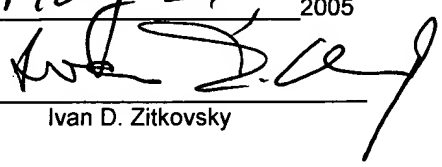

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